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Evidence for High Genetic Diversity and Long-Term Endemicity of Hepatitis C Virus Genotypes 1 and 2 in West Africa

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During 1994 and 1995, the prevalence of hepatitis C virus (HCV) and its genotypes were studied in several rural and urban populations in three West African countries: Guinea, Burkina Faso, and Benin. The following groups were screened for antibodies to HCV (anti-HCV): 459 villagers in the forest region of Guinea; 965 individuals in urban, suburban, and rural populations of the Bobo Dioulasso area, Burkina Faso; and 582 blood donors in Cotonou, Benin. In Benin, 60 patients with sickle cell anemia (30 with and 30 without history of multiple transfusion) and 13 hospital patients with liver disease were also tested. RT-PCR detection of HCV-RNA was carried out on all anti-HCV positive samples, followed by genotyping and sequencing of unrecognized subtypes. The prevalence rates of anti-HCV were 1.1% in the Guinean population group, 1.4% among blood donors in Benin, and 4.9% in residents of Burkina Faso. In patients with sickle cell anemia, five of the 30 polytransfused patients (17%) had anti-HCV, whereas none of the patients without a history of blood transfusion had anti-HCV ($P < 0.05$). Among the 13 patients with liver disease, five had anti-HCV, of whom four had history of blood transfusion. HCV-RNA was detected in 41 anti-HCV positive sera. All belonged to genotypes 1 or 2, with a high genomic diversity; 18 different subtypes were identified, including 2c, 2d, and 16 new subtypes. Such genetic diversity poses a challenge for vaccine development and also implies that HCV infection is long-established in these West African regions. ***J. Med. Virol. 55:92–97, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: hepatitis C virus; West Africa; epidemiology, genotypes; liver disease; transmission

INTRODUCTION

Hepatitis C virus (HCV) infection has been reported in West Africa [Denis et al., 1994; Develoux et al., 1995; Mellor et al. 1995; Mutimer et al., 1994], although data on its distribution in populations are scarce. Very little is known about the genotypes involved. Recently, a new subtype 2d of genotype 2 was isolated from a Nigerian patient treated in the Netherlands [Stuyver et al., 1994] and a new subtype of genotype 1 was also characterized in a serum sample from Nigeria [Mellor et al., 1995]. An additional genotype 2 subtype was identified in two samples from the Gambia [Mellor et al., 1995]. This suggests a different distribution of HCV genotypes in West Africa compared with Central Africa, where genotype 4 largely predominates [Fretz et al., 1995; Xu et al., 1994].

A series of surveys was carried out to investigate the

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prevalence of HCV infection and the genotype distribution in several rural and urban general populations in three West African countries: Benin, Burkina Faso, and Guinea. In Benin, patients with liver disease and patients with sickle cell anemia were also tested. Polymerase chain reaction (PCR) detection of HCV-RNA and genotyping were carried out on all sera with antibodies to HCV (anti-HCV).

MATERIALS AND METHODS

Study Populations

In the forest region of Guinea, a large proportion (estimated coverage 70%) of the population over 15 years of age in a village of the Guerzé ethnic group and a village of the Mano ethnic group were screened for anti-HCV during November and December of 1994. The number of individuals tested was 203 in the Guerzé village and 256 in the Mano village. Due to the small quantity of blood taken, subsequent PCR detection of HCV-RNA was not possible. Later, in May and June of 1995, blood samples were taken from 43 families (172 individuals), including three with a member positive for anti-HCV, with the aim of characterizing the HCV genotypes in this area. In the Bobo Dioulasso area of Burkina Faso, the following study populations were tested for anti-HCV during the period from November 1994 to July 1995: 327 consecutive outpatients from the urban population undergoing blood tests at the laboratory of the Muraz Center, 277 individuals from a suburban area, and 361 from a rural village. These last two groups included the families involved in a prospective malaria survey, selected randomly from a large sample of volunteers.

In Benin, 582 consecutive blood donors, during a two-week period in February and March of 1994 at the University Hospital of Cotonou, were tested for anti-HCV. In addition, anti-HCV testing was carried out in 30 consecutive polytransfused patients with sickle cell anemia, selected during the period from November 1994 to March 1995, and in 30 consecutive patients with the same disease who had not received any blood product. Polytransfused patients were those who had received blood products at least three times. During the same period, a sample of 13 patients presenting with severe hepatic diseases were investigated; liver function tests and ultrasound, serodiagnosis of hepatitis B virus surface antigen (HBsAg), and anti-HCV were undertaken to establish a diagnosis.

In these three countries, permissions from health authorities as well as informed consent from all individuals included in these surveys were obtained, except for blood donors and outpatients where screening was anonymous.

Serological Diagnosis

All sera were screened for anti-HCV by ELISA. Monolisa HCV (Sanofi Diagnostics Pasteur, Marne-la-Coquette, France) was used for all groups except blood donors in Cotonou, Benin, where HCV ELISA second generation (ORTHO Diagnostic Systems, Raritan, New Jersey) was used. All ELISA positive samples were further tested by two immunoblot assays Deciscan (Sanofi Diagnostics Pasteur) and INNO-LIA (Innogenetics, Ghent, Belgium).

RNA Detection and Genotyping

All sera with positive or indeterminate results by immunoblot were tested for HCV RNA by a standard RT-PCR procedure [Stuyver et al., 1995]. HCV genotype was determined by the INNO-LiPA HCV II (Innogenetics), which allows discrimination of genotypes 1 to 6 on 5′ untranslated region (UR) amplified fragments. When a positive amplicon of the 5′-UR/Core region was obtained, genotyping was confirmed by a 5′-UR/Core LiPA for genotypes 1 and 2. Direct sequencing of the 340-bp NS5B region was achieved as described [Stuyver et al., 1995]. Based on previous studies, amplification primers for the 5′-UR/Core region were optimized. Sequences obtained during the course of this study will appear in the GenBank under accession numbers AF037230–AF037254.

Data Analysis

Prevalence estimates were obtained by calculating the percentages of anti-HCV positive individuals in general populations and blood donors. Chi-square tests, indirect standardization of prevalence rate, and calculation of a standardized morbidity ratio (SMR) were carried out as appropriate. Fisher's exact test was used when small numbers were involved.

To identify new subtypes, comparison with published sequences (NS5B region) was achieved using the phylogenetic software PHYLIP, and sequences were presented in a sequential format to the DNADIST program (Kimura-2 parameter). The distance matrix was further interpreted by the NEIGHBOUR program (UPGMA settings). Segregation into subtypes was carried out using a border value obtained from previous studies [Stuyver et al., 1994].

RESULTS

Prevalence of Anti-HCV in General-Population Groups in Guinea and Burkina Faso

In the forest region of Guinea, 0.8% of Mano villagers and 1.5% of Guerzé villagers had anti-HCV (Table I). In the Bobo Dioulasso area of Burkina Faso, the prevalence of anti-HCV was 2.2% in the suburban population and 3.9% among rural villagers. In all these population groups, the prevalence rates in men and women were similar (P value for difference > 0.10).

Prevalence of Anti-HCV in Blood Donors and Unselected Clinic Outpatients

In the Bobo Dioulasso area of Burkina Faso, 8.3% of urban outpatients were anti-HCV positive. The apparent difference in prevalence between the urban outpatients and the general-population groups described above was in part due to the small number of children (15 aged younger than 15) among outpatients. Using the age-specific rates found in the general-population

TABLE I. Prevalence of Anti-HCV in Different Groups in West Africa

Population	Number tested	Gender		Age mean (SE)[a]	Anti-HCV positive			P
		Male	Female		n	%	95% CI	
Guinea (forest region, villagers)								
Mano ethnic group	256	41%	59%	36.8 (1.1)	2	0.8%	(0.1–2.9)	0.66[b]
Guerzé ethnic group	203	31%	69%	37.6 (1.0)	3	1.5%	(0.4–4.6)	
Total	459				5	1.1%	(0.1–2.1)	
Burkina Faso (Bobo Dioulasso area)								
Urban outpatients	327	58%	42%	37.0 (0.8)	27	8.3%	(5.3–11.3)	.001[c]
Suburban area	277	43%	54%	24.2 (1.1)	6	2.2%	(0.6–3.8)	
Village	361	44%	56%	22.3 (0.9)	14	3.9%	(1.9–5.9)	
Total	965				47	4.9%		
Benin (Cotonou, University Hospital)								
Blood donors	582	85%	15%	27.0 (0.3)	8	1.4%	(0.4–2.4)	
Patients with sickle cell anemia								
Multiple transfusions	30	43%	57%	18.5 (2.0)	5	17%	(6.5–35.5)	.03[b]
No transfusion	30	50%	50%	15.2 (1.7)	0	0%	(0.0–14.1)	
Patients with liver disease	13	62%	38%	44.1 (5.2)	5	38%	(15.1–67.7)[b]	

[a]SE = standard error.
[b]Fisher's exact test and exact CI limits.
[c]Chi-square test.

groups (age groups 0–14, 15–29, 30–44, 45 and over), the expected rate in urban outpatients was 5.0%. The corresponding SMR was 1.7 (95% CI; 1.1–2.4), significantly higher than 1 ($P < 0.02$). In Benin, the prevalence in blood donors, mainly young males, was 1.4%. There was no significant difference between men and women in either group.

Prevalence of Anti-HCV in High-Risk Patients in Benin

In patients with sickle cell anemia, five out of the 30 polytranfused patients (17%) had anti-HCV. In contrast, none of the 30 patients who had not received blood products had anti-HCV ($P < 0.05$).

The 13 patients with liver disease included: six cases of cirrhosis, one of hepatocellular carcinoma, five of acute hepatitis, and one of chronic hepatitis. Anti-HCV were found in five (38%) of these patients, including four of the six cases of cirrhosis, and one of the six cases of hepatitis. Among the five anti-HCV positives, two were coinfected with HBV (i.e., HBsAg carriers), and four additional patients were infected with HBV only. In these five anti-HCV positive patients, four had a history of blood transfusion, while none of the anti-HCV negative patients had been transfused.

HCV-RNA Detection and Genotypes

Overall, 69 anti-HCV positive serum samples from the different groups described above were tested by RT-PCR, and 41 (59%) were HCV-RNA positive (Table II). Among the samples stored in good conditions (see footnotes of Table II), HCV-RNA was detected in 82% of anti-HCV positive samples. In addition to the anti-HCV positive samples, 31 samples with indeterminate results by the immunoblot assay Deciscan (Sanofi Diagnostics Pasteur) were also tested by RT-PCR; none were positive. Of the 41 HCV-RNA positive samples, 17 (41%) were genotyped as type 1 and 24 (59%) as type 2. The three countries did not differ significantly regarding the relative frequencies of genotypes 1 and 2 (Fisher's exact test, $P > 0.10$).

Characterization of New Subtypes

Using genotype-specific Core antisense amplification primers, a 5'-UR/Core amplicon could be obtained from 29 HCV-RNA positive samples and was genotyped using type 1 and type 2 5′-UR/Core LiPAs. Three samples from urban outpatients in Burkina Faso were closely related to subtype 2c, the most prevalent subtype of genotype 2 in Europe. However, the 26 remaining samples could not be classified into 1a, 1b, or 1c subtypes, nor in 2a, 2b, or 2c subtypes. The NS5B region was then sequenced from 25 of these samples, including nine samples in genotype 1 and 16 samples in genotype 2. Phylogenetic analysis revealed that four isolates from Benin belonged to subtype 2d, recently characterized from a patient originating from neighboring Nigeria [Stuyver et al., 1994]. The other samples were classified as belonging to five new subtypes of genotype 1 and 11 different new subtypes of genotype 2, as shown in Table II. The phylogenic tree (Fig. 1) clearly shows the different new branches for type 1 and type 2. Branches not labeled with a subtype are new candidates. In order not to further confuse the variability field, no subtyping assignment is given. However, all these unlabeled branches fulfill the criteria for subtypes as established previously [Stuyver et al., 1994]. In conclusion, 18 different subtypes were found in these West African areas, including 2c, 2d, and 16 new subtypes.

DISCUSSION

HCV infection was found in all groups tested in the three West African countries surveyed, including both

TABLE II. Detection of HCV-RNA in Anti-HCV Positive Samples by PCR and Genotyping

Population	Number tested	RNA positive, PCR positive 5′ UR		HCV genotype, 5′ UR amplicon		PCR positive 5′ UR/core, n	HCV subtypes[a]	
		n	%	Type 1	Type 2		Type 1	Type 2
Guinea (forest region)							3 isolates	1 isolates
Villagers	4	4	100%	3	1	4	1 new (n = 3)	1 new (n = 1)
Burkina Faso (Bobo Dioulasso area)							4 isolates	13 isolates[c]
Urban Outpatients	27	23	85%	8	15	18	3 new (n = 4)	2c (n = 3)
Suburban area	6	0	0%[b]	n.a.	n.a.	0		9 new (n = 12)
Village	14	3	21%[b]	1	2	0		
Total	47	26	55%	9	17	18		
Benin (Cotonou)							2 isolates	5 isolates
Blood donors	8	4	50%[d]	1	3	2	1 new (n = 2)	2d (n = 4)
Patients	10	7	70%	4	3	5		1 new (n = 1)
Total	18	11	61%	5	6	7		
Total	69	41	59%	17	24	29	9 isolates 5 new (n = 9)	19 isolates 2c (n = 3), 2d (n = 4) 11 new (n = 12)

[a]Genotyping by 5′-UR/Core LiPA and confirmed by NS5B sequencing for new subtypes. The number of new subtypes characterized in each country is given. The number of isolates belonging to a previously known subtype or to one of the new subtypes is given in parentheses.
[b]These blood samples were collected in heparinized tubes for a malaria survey; this explains the low percentage positive by RT-PCR.
[c]The NS5B region could not be amplified for one isolate unclassified by 5′-UR/Core LiPA.
[d]Irregular conditions of cryopreservation could decrease the number of HCV-RNA positive samples.

rural and urban populations, blood donors and hospital patients. All 41 PCR positive samples belonged to genotype 1 and 2, but displayed a surprisingly large genomic diversity: 18 different subtypes were found, including 16 new subtypes. Such genetic diversity implies the long-term presence of HCV infection in these West African regions, particularly of genotypes 1 and 2 [Mellor et al., 1995].

To estimate prevalence rates, not only were blood donors and outpatients studied, but also groups drawn from the general population. Blood donors compose a highly biased groups in Africa, with young age and male predominance, and outpatients were older than the general population. The results therefore point to the widespread presence of HCV infection in West Africa both in rural and urban populations. As the areas from which our population samples were drawn were not randomly selected, the data do not necessarily provide an accurate estimate of the prevalence of HCV infection in an entire region or country. In this study, the specificity of the serodiagnosis was confirmed by the high rate of HCV-RNA positivity when sera were properly stored, and the proportion was very similar to that observed in industrialized countries.

The data on selected groups of patients in Benin, although based on small numbers, demonstrate the role of blood transfusion in HCV transmission in urban areas, and suggest that patients with sickle cell anemia are at high risk of HCV infection, as reported also in Nigeria [Mutimer et al., 1994]. However, blood transfusion cannot account for a long-standing presence of HCV in these regions, nor for most of the cases found in the rural populations, where a history of blood transfusion is very rare [Fretz et al., 1995]. Consequently, HCV transmission routes in these population have yet to be identified, both to explain the endemicity of HCV and to develop preventive strategies. The rate of vertical transmission is low, as established by recent studies [MacDonald et al., 1997; Ohto et al., 1994]. Although sexual transmission rates are far from well documented, sexual transmission is possible, with a low efficiency [MacDonald et al., 1997].

A recent report describing the phylogenetic analysis of seven HCV-RNA positive samples from a survey in Guinea, from hospital in- and outpatients in Conakry, is consistent with our findings: genotypes 1 and 2 are predominant in West Africa, with considerable genomic diversity [Ruggieri et al., 1996]. Moreover, subtypes 1a 1b, 2a, and 2b, which are common in the industrialized world, were not encountered in West Africa. From present knowledge of HCV genetic diversity worldwide, two patterns have emerged. The first is the recent epidemic spread that took place during the last 50 years in Europe, North America, Japan, and Australia, with a limited number of genotypes, mainly 1a, 1b, 2a, 2b, 2c, and 3a, and related to the parenteral pathways, e.g., use of blood products and intravenous drug abuse [Davidson et al., 1995; Mellor et al., 1995; Pawlotsky et al., 1995]. The second pattern is a long-term endemicity, allowing the emergence of many different subtypes over the time, although the transmission routes explaining such a pattern are as yet unclear. Such endemic spread appears to have taken place in the Indian subcontinent with type 3 [Mellor et al.1995], in Southeast Asia with types 6 to 11 [Tokita et al., 1996], in Central Africa with type 4 [Mellor et al.,

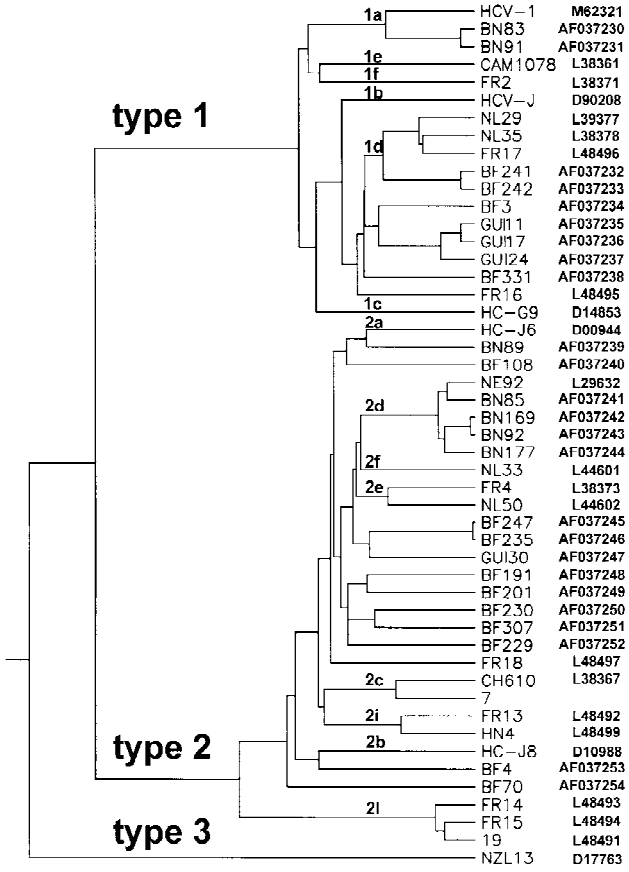

Fig. 1. Phylogenetic tree of the HCV NS5B region (nt positions 7935–8274). Subtypes are indicated where relevant. Accession numbers obtained during the course of the study are indicated as AF037230–AF037254. Samples from Guinea are abbreviated as GUI, from Burkina Faso as BF, and from Benin as BN.

1995; Stuyver et al., 1994], and, as demonstrated here, in West Africa with types 1 and 2. Such genetic diversity poses a severe challenge for vaccine development, since subtypes differ by ca 20% of the amino acid sequences in the envelope regions.

Several studies in Europe, Japan, and North America had a poorer response to interferon alpha treatment, a faster progression and greater recurrence after liver transplantation for infection with HCV subtype 1b [Gane et al., 1996; Kobayashi et al., 1996; Simmonds et al., 1996; Takada et al., 1996; Zein et al., 1996]. However, in most of these studies, the duration of infection and the age at infection were not taken into account. A recent report summarizing the natural disease progression in a large series of patients in France found no significant effect of subtypes 1a, 1b, 2a, 2 b, 2c, and 3a after adjusting for age at infection and other factors [Poynard et al., 1997]. Whatever the underlying cause of the observed association between HCV subtype and disease outcome, genotyping is still a valuable prognostic marker in clinical practice, as age at infection is often unknown. Furthermore, the pathogenicity and response to treatment of the subtypes found in Southeast Asia and Africa have not yet been investigated.

In West Africa, cirrhosis and liver cancer are a major cause of mortality in males, a large part attributable to HBV infection. The contribution of HCV is not yet established, but the preliminary findings reported above from patients with liver disease in Benin and other reports [Skalsky et al., 1995] suggest that HCV does cause liver disease in Africa and point to blood transfusion as a frequent route of transmission in urban populations [Mutimer et al., 1994]. Therefore, HCV infection in Africa is a public health concern. To ensure the safety of blood products, the promotion of nonblood replacement therapies and the control of nosocomial risk of transmission are urgent measures needed in this region, as they are in other areas of HCV endemicity in developing countries. In industrialized countries, a number of documented cases of HCV transmission within hospital communities through contaminated material such as dialysis equipment [Allander et al., 1994], and through other unrecognized routes of patient-to-patient transmission [Allander et al., 1995], have been reported. The standards of disinfection and sterilization and the quality of equipment are far lower in most developing countries. For example, recycling of single-use disposable materials is commonplace due to financial and supply constraints. Hence, the risk of occupational and nosocomial infection in HCV endemic areas is obviously increased [Volkow et al., 1997].

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